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(57) Abstract

This invention allows for the oral, parenteral or inhalation delivery of large macromolecules. Biologically active drugs entrapped into biodegradable hydrogel polymers in either organic and water phase systems. By using cyclodextrins, sensitive molecules can be protected during the granulations of nanoparticles production phase.

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DRUG DELIVERY SYSTEMS FOR MACROMOLECULAR DRUGS

FIELD OF INVENTION

The present invention relates to improved pharmaceutical formulations for administration of macromolecules or drugs that are not bioavailable using standard pharmaceutical methods. More specifically, the invention relates to pharmaceutical formulations of controlled release rate dosages of the drugs that may be administered orally, parenterally, or by inhalation.

BACKGROUND OF THE INVENTION

Oral delivery of drugs or long acting parenteral dosage forms of drugs is preferred by the patient and physician because of compliance and the inherent beneficial effect of constant pharmacodynamic action. Fundamentally, for a drug to be absorbed by the intestine it must first become soluble in the aqueous media of the gut. Products that are rapidly dissolved in water are usually rapidly absorbed into the body. For such products, controlling the rate of solvation or rate of absorption after ingestion also influence their bioavailability and subsequently their pharmacodynamic effectiveness.

Proteins and polypeptides, as well as some complex carbohydrates, however, are degraded by enzyme systems in the intestinal tract and are thus not orally active. Similarly these drugs when given parenterally may have short half lives or may not have effective injection delivery systems. This problem arises from the fact that the drugs are destroyed before being absorbed or they are of such a size and nature that the body cannot absorb the medication. Similarly, their injectable forms have short durations of action, requiring frequent injections, making the products unsuitable for use in the non hospitalized patient.

It is medically desirable to deliver medicinal agents to the body in a smooth and controlled fashion. This avoids the "peaks and valleys" of most immediate release oral and parenteral preparations. Common factors influencing drug absorption is the passage of drugs across the intestinal membrane. A drug almost always has to cross several cellular membranes to reach its receptor site. This transfer across cell membranes is normally accomplished by passive diffusion. Special transport mechanisms such as facilitated diffusion and active transport allow some substances to cross cell membranes at a faster rate than simple diffusion. By far the most common procedure for drug transport across the cell membrane, however, is passive diffusion.

Passive diffusion is characterized by the movement of a drug molecule down an electromagnetic or concentration gradient without the expenditure of cellular energy. The transfer process is neither saturable nor inhibited by other materials and is only slightly sensitive to temperature changes. Since most cells in the gastrointestinal tract are in proximity to capillaries, the passage of drugs across this short distance is usual rapid.

The driving force for passive drug diffusion is the difference between the concentration gradient of the diffusing drug in the intestinal tract and the concentration gradient on the other side of the plasma membrane. The rate of drug penetration-diffusion corresponds to the concentration gradient as is characterized by Fick's law.

Many drugs are either an organic acid or a base. Acids donate a hydrogen to form a negatively charged anion while bases accept a hydrogen ion to form a positively charged cation. It is usually assumed that only nonionized, lipid-soluble drugs pass through the lipid rich membranes of the intestinal tract. The ionized molecules are thought to be too polar to penetrate this lipoid barrier. If the ionized molecule does cross the cell wall, however, it does so at a slow rate. This concept of drug absorption is known as nonionic diffusion.

An extension of this principle is the pH partition hypothesis, which states that the passage rate of a drug through a membrane is dependent upon the pH of the drug's environment and the dissociation constant, or pKa of the drug. The pKa is expressed as that pH at which 50% of the drug will be ionized and 50% will be in the nonionized form. Diffusion of acids and bases across the cell membrane is not always influenced by pH, as in the case of weak acids or bases. These types of products are essentially completely nonionized at all physiological pH values. At the other extreme, however, are strong acids and bases that are almost completely ionized, and their transfer is dependent upon the pH at which they become dissolved and subsequently become ionized or nonionized.

An example of this pH partition hypothesis may be explained by the fact that aspirin, which has a pKa, or dissociation constant of between 3-3.7, becomes very nonionized in the acid media of the stomach and subsequently is rapidly absorbed from the gastric mucosa, where the pH is between 1-3. As the drug particles pass into the small intestine where the pH increases, the rate of ionization is changed so absorption is subsequently slowed. Conversely, strong bases such as ephedrine, which has a pH of

9.3, are almost negligibly absorbed in the acid media of the gastric juice and are absorbed rather rapidly from the intestinal media which has a much higher pH.

While the pH partition hypothesis is but one of the factors influencing drug absorption, it cannot explain all the phenomena observed with different delivery systems and the rate of drug delivery in such systems.

SUMMARY OF THE INVENTION

The present invention obviates the problems of solvation as well as the need for classic absorption for delivery of those drugs where the molecular size is too large to be absorbed when the product is formulated and administered, or because of the deleterious effects of gastrointestinal enzymes, and other factors. The invention is directed to pharmaceutical formulations for effective controlled release of many drugs not now orally or parenterally available. These formulations, properly adjusted, also may be administered by inhalation and achieve the same kinetic characteristics.

The pharmaceutical formulations of this invention are made by entrapping the drug of choice in either an organic or water phase biodegradable hydrogel polymer system to produce nanoparticles. These nanoparticles are then coated or combined with one or more bioadhesive adjuvants to promote adherence of the particles and their medications to the intestinal wall.

There are two preferred pharmaceutical formulation embodiments to form the nanoparticles: In the first instance, drugs that are rugged and can withstand organic solvents are treated by entrapment in single or combinations of biodegradable hydrogel polymers. Alternatively, non-organic solvents may be used, but it is preferred to entrap drugs that need a water-based system in a cyclodextrin. This entrapped drug nanoparticle complex may then secondarily be entrapped in liposomes.

With those drugs that are delicate to changes in their environment, the drugs can first be entrapped into a cyclodextrin for protection, then granulated with water soluble, hydrogel polymers. These entrapped cyclodextrin-drug complexes can be secondarily entrapped in liposomes before or after their enclosure into the hydrogel. The invention is therefore suitable for using both organic and non-organic solvents.

The advantage of the nanoparticle system is that its absorption by the body is by the lymphatic or lacteal system. Because of the resistant hydrogel coating it is not affected by the enzymes or degradative influences present in the gastrointestinal tract. Using bioadhesive hydrogel polymers ensures a more prolonged duration of time in which the nanoparticles are in contact with the intestinal mucosa, obfuscating the

deleterious action of heightened gastrointestinal peristalsis. All of these compounds work in harmony to produce viable products that have demonstrated favorable and reproducible effects in animals.

The invention thus describes simple and predictable methods for the preparation of oral, parenteral, or inhalation dosage forms of a drug or drugs entrapped in biodegradable hydrogel polymers. The following combinations may be used to formulate a controlled release pharmaceutical product:

- A controlled release pharmaceutical formulation with a biologically active molecule which is formed into nanoparticles with biodegradable hydrogel polymers and then coated with one or more bioadhesive adjuvants.
- A controlled release pharmaceutical formulation with a biologically active molecule which is formed into nanoparticles with biodegradable hydrogel polymers, coated with liposomes, and then coated with one or more bioadhesive adjuvants.
- A controlled release pharmaceutical formulation with a biologically active molecule encapsulated with a cyclodextrin, which is then formed into nanoparticles with biodegradable water-soluble hydrogel polymers, and then coated with one or more bioadhesive adjuvants.
- A controlled release pharmaceutical formulation with a biologically active molecule which is encapsulated with a cyclodextrin, entrapped with liposomes and formed into nanoparticles then with water-soluble hydrogel polymers, and then coated with one or more bioadhesive adjuvants.

A controlled release pharmaceutical formulation with a biologically active molecule encapsulated with a cyclodextrin that is then formed into nanoparticles with biodegradable water-soluble hydrogel polymers, coated with liposomes, and then coated with one or more bioadhesive adjuvants.

The sphere size in the nanoparticle will typically be in the range of 500 to 1500 nm, so that the drug(s) so entrapped may be administered orally, parenterally, or by inhalation. The exact diameter of the nanoparticles is not critical, provided that is sufficiently small for cell diffusion by the lymphatic or lacteal system.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the chemical nature of the cyclodextrin skeleton.

Figure 2A is a photomicrograph of a scanning electron microscope at 30,000X magnification, showing size and diameter of heparin nanoparticles. Figure 2B is a

photomicrograph of a scanning electron microscope at 6,000X demonstrating clusters of nanoparticles.

Figure 3 is a graphic display comparing the levels of plasma heparin from Table 1. This figure shows the mean (±SD) plasma heparin values for 12 rabbits given oral heparin nanoparticles plus adjuvant (2,400 U/kg), and for 1 rabbit each given oral heparin (11,000 U/kg), oral heparin (11,000 U/kg) plus adjuvant, or adjuvant alone.

DETAILED DESCRIPTION OF THE INVENTION

Simple and predictable methods have been discovered for the preparation of controlled release active dosage formulations of drugs entrapped in biodegradable hydrogel polymers, with alternative concomitant entrapment in a protective quantity of cyclodextrins. The polymer/drug complex and the polymer/drug/cyclodextrin complex may then be coated with phospholipids or other lipoid components forming secondary liposomes. These combinations are then combined with an appropriate quantity of bioadhesive adjuvant. The drugs so entrapped may be administered orally, parenterally, or by inhalation.

The rate of active drug release from the entrapped spheres is dependent upon the basic kinetics of the drug administered, the amount of hydrogel polymer, the cyclodextrin used, the phospholipid coating and, in the case of parenteral administration, the method of administration, the area of deposition, and the vascularity of the body region. With inhalation dosing, it is not necessary to use the bioadhesive adjuvants, although the adjuvants may be included in these formulations.

The invention is thus directed to controlled release rate formulations of biologically active molecules, especially macromolecules. Any therapeutic drug, biological active protein or polypeptide, polysaccharide molecule, carbohydrate complex vaccine, such as an influenza vaccine, and the like, referred to generally herein as "bioactive material," "biologically active molecule," or "drug," can be formulated according to this invention. In addition, more than one drug can be formulated together according to this invention.

Primary candidates for development into oral formulations are the widely used drugs, particularly those drugs with low bioavailability and drugs currently only capable of being administered via parenteral means. Two of the preferred drugs that can be prepared according to this invention are heparin and insulin. These drugs are classic large molecular weight drugs that have been considered the gold standard for effectiveness of new drug delivery systems for large polypeptide or carbohydrate drugs,

and have previously been unavailable by oral means. With the pharmaceutical formulations of this invention, these drugs are not only absorbed orally, but their pharmacological effects are prolonged.

Heparin is a well-established drug that is a complex polysaccharide that is highly charged (electronegative). It has a molecular weight of 20,000-60,000 daltons, which are covalently attached to a core protein found in most secretory cells. Heparin is a multifaceted drug with primary actions as an anticoagulant. When venous or arterial thrombi occur and the patient survives, heparin is given at doses of 10,000-20,000 units every 4-6 hours to maintain blood levels at 0.5-1.5 anti-factor Xa units/mL, which should prevent further thromboembolic phenomena. After this, the heparin dose is usually maintained at 10,000 units every 4-6 hours to prevent further thromboembolic events.

In addition, heparin has the ability to bind to the arterial wall following angioplasty and ameliorate the proliferation of smooth muscle cells and ameliorate the restenosis that so often occurs with this procedure. Also, heparin is used as a preventive agent for those patients that are at close risk of stroke or heart attack as well as the patients recovering from a heart attack.

Heparin also has a clearing effect in the blood by activating lipoprotein lipase on the cell surface. This action clears hyperlipoproteinmia and lowers the low density lipoprotein. In animals, it has reversed the athrogenic deposits on the arterial walls, which is a phenomenon of arteriosclerosis in humans.

Another of the drugs that has been previously studied for oral administration is insulin. Insulin is a polypeptide with two peptide chains linked by a disulfide bridge. The A chain of insulin contains 21 amino acids and the B peptide chain contains 30 amino acids. This structural integrity is important for its biologic activity. If the amino acid sequences are disrupted or the disulfide bridge broken, the hormone becomes inactivated.

According to the present invention, two methods are provided for incorporating bioactive molecules in nanoparticles. The first method covers materials that are soluble in organic solvents; the second method is for those molecules that are water soluble only.

BIODEGRADABLE HYDROGEL POLYMERS

Microparticles (<900 nm - 10 nm) have been used for a number of years as a drug delivery method. This is when hydrogel polymers are allowed to entrap an organic molecule, then fixated it into spheres, by several methods. This allows the drug to enter the body protected from hazardous enzymatic digestion in the gastrointestinal tract. In

general, the spheres are produced by dissolving the active medicinal agent in a suitable solvent such as buffered water or purified water. The hydrogel, which are polymers of biodegradable compounds, are most often soluble in organic agents such as dichlomethane, acetone, and the like. The dispersed polymers are solvated and then added to the water-drug combination and spheres of micro particle size are formed. These spheres, entrapped in biodegradable material, are somewhat immune to the deleterious effects of the gastrointestinal enzyme. Since they are biodegraded, they can be properly formulated so that oral and parenteral administration is possible.

Hydrolysis of the polymer breakdown is most desirable since this will produce low molecular weight by-products. Some of the more common biodegradable polymers are: poly (lactic acid), poly (glycolic acid), (poly (e-caprolactone), poly (p-hydrohybutyrate), poly (b-hydroxyvalerate). poly (tartonic acid), polydixamone, poly (ethylene terephalate) poly (ortho esters), polyanhydrides, dextran, cross-linked polyvinyl alcohol, polyhydroxy methacrylate, polycyanoacrylates, poly (phosphoesters), polyphosphgenes, poly (hydroxy butyrate co-valerate), poly (2-hydroxyethyl glutamate), polyvinylpyrrolidone, poly (ethylene glycol), poly (propylene glycol), chitosan, pullulan, zein, alginic acid and alginate salts.

In addition, other methacrylate polymers can be used specifically in water soluble systems. These include, but are not limited to acrylic acid, methacrylacetic cyanoethyl methocrylitic aminoakyl methacrylate copolymers, ethoxyethyl methacrylate copolymers, polymethacrylic acid, methacrylate acid alkylamide copolymer, polymethacrylic acid, polyacrylic acid, methacryic acid alkylamide copolymer, polymethacrylic acid (anhydride), and polymethyl methacrylate. The advantage of the acrylate series is that when properly compounded they are water soluble or can be supplied as a 30% aqueous solution with varying degrees of solubility.

Other polymers known to the art can be used in either the water or organic phase, if properly prepared. An example of this is alginic acid in intimate admixture with the drug, then cross-linking the alginic acid with a known inorganic element such as zinc, magnesium, calcium, or sodium salts. Likewise pectin, zein, and guar gum can be used and cross-linked with a metallic ion. Ethyl cellulose can also be used as a polymer to entrap the molecule and can be used in an aqueous or organic solvent system. Similar examples are polyvinyl acid phthalate, cellulose acid phthalate, cellulose acid trimaleate phthalate and similar cellulosic polymers such as hydroxypropyl methyl cellulose phthalate and the like.

CYCLODEXTRINS

Cyclodextrins are a new class of compounds derived from corn. They are cyclic, non-reducing oligosaccharides built up from six, seven, and eight glucopyranose rings known respectively as alpha, beta, and gamma cyclodextrins. In addition, hydroxypropyl and other groups have been and are added to the molecule giving each series its own specific characteristics and pharmacological behavior. These molecules have a unique shape as shown in Figure 1. The cyclodextrins act as a biodegradable trapping agent, incorporating the active medication into its core and forming a unique molecular inclusion complex. These complexes provide an anchoring compound with the formation of covalent bonds. This guest-host complex protects the active drug, while not forming tight chemical bonds.

With the drug in a buffer or suitable solution and, with agitation, cyclodextrins can be used to entrap the drug, providing a guest-host interaction. Cyclodextrins are usually used in amounts that range from 0.25%-2% W/W, depending upon the bioactive molecule and the degree of protection required. The type of cyclodextrins used will again depend upon the molecule and the degree of protection required. These parameters can easily be determined by routine experimentation by one of skill in the art.

LIPIDS AND LIPOPROTEINS

Liposomes were first characterized in 1965. They are single or combinations of lipids formed into spherical shapes with drugs or other active medicaments. Lipids or liposomes, primarily phospholipids, are, in general, readily absorbed intact by the intestinal cells, degraded as they pass through the interstitial space and slowly reconstituted by the body where they are then excreted by active transport in the ileum and colon in a newly reconfigured formulation. About 30-40% of the fecal dry weight is fat or lipid. Most importantly, essentially all the fat or lipids ingested are absorbed directly into the portal blood and lymphatic system or by transcell migration. Liposomes, like their parent lipids, can enter the system in a number of ways:

1. Intermembrane transfer, which occurs when liposomes are in close contact with the cell they are exchanged with certain lipids in the cell wall, where they are transported intact into the cell.

2. Contact release, which occurs when liposomes contact the cell and there is a sudden permeability of the lipid to the interior of the cell.

3. Absorption, which occurs when there is electrochemical attraction between the cell and the liposomal particle, wherein the particle is pulled into the cell.

4. Fusion, which occurs when there is a close approach of the lipids in the cell wall and those of the liposomal lipids. Fusion results in complete mixing of the plasma membrane and release of the liposomal contents into the cytoplasm of the cell.

5. Cells with phyacytic capability take liposomes into endosomes, (which are intracellular molecules formed by invagination of the plasma membranes). Endosomes are then degraded into their component parts.

While liposomes have been used for some drug delivery, there are problems with manufacturing, stability, and assurance of release. Their advantage is that drugs encapsulated into a lipid sphere can carry an electrostatic charge, allowing them to interact with cells of the opposite charge. By such a process of fusion and constant release, they efficiently can deliver the active drug moiety to the body.

When liposomes are used as a secondary granulating agent, the preferred agents are cholesterol, lecithin, glycerol monostearate, phospholipon 90H (Natterman) maleated soybean oil, sphingomylein, phosphatidyl entanolamine, dipamitoyl phoisphophatidykaline, phophosinositol, phophatidic acid, triglycerides. Since lipids tend to undergo oxidative degeneration, alpha tocopherol is usually added as an ancillary agent along with other antioxidants when the drug and or the liposomes.

BIOADHESIVE ADJUVANTS

The preferred bioadhesive adjuvant(s) that is added only to the total formulation are hydroxypropyl methyl cellulose, methyl cellulose, pectin, guar gum, xantham gums, gum acacia, gum dragon, hydroxypropyl alginate, sodium carboxymethyl cellulose, carbomer 934-P, acrylic acid derivatives and those of similar pharmaceutical characteristics and behavior. These adjuvants are pharmacopeial items and are blended in with the nanoparticle granulate at the final stage of production. The preferred dose is a single or combination of these adjuvants at a 50/50 W/W ratio and a percentage weight of the total granulate of 0.1-3% preferably in the range of 0.3-1.5% and most preferably 0.2-1.2 weight percentage.

The invention describes the formulation of a range of products with a variety of process steps and combinations. Embodiments in addition to those illustrated will be readily understood by those skilled in the art. The present invention includes within its

scope novel processes and products derived from the invention whether as individual features or in combination with each other to produce novel combinations. The invention is illustrated further by the following examples, which are not to be taken as limiting in any way.

EXAMPLES

In the examples that follow, gastrointestinal absorption of oral formulations of water soluble dyes entrapped into nanoparticles, prepared according to the present invention, were studied by light microscopy. Nanoparticles containing fluorescent stains were prepared and administered orally with concomitant bioadhesive adjuvants, as a single dose to anesthetized rabbits, via a gastric tube. The rabbits were sacrificed 7 and 14 days after oral administration of the manufactured spheres. Both ultraviolet light microscopy and direct vision revealed dye-containing spheres widely distributed throughout the animal's bodies.

Microscopic observation of the tissue distribution of the dye at various times following a single oral administration of florescent containing nanoparticles suggested that absorption probably occurs by pinocytosis, as well as by direct lacteal absorption and distribution. Pinocytosis, an active transport process by which small particles of molecular or macromolecular dimensions are incorporated into intracellular vacuoles that originate by the folding of the plasma membrane around the material being engulfed, was also active.

Example 1

Formation of Heparin-Containing Nanoparticles

300 mg of heparin sodium (1mg=157 units) derived from porcine intestines, MW ~25,000 daltons, was weighed and blended in a high shear mixer with 50 mL of purified water.

To make the nanoparticles, 600 mg of Poly(3-hydroxybutyrate 3-covalerate (80:20) and 300 mg of DL-Lactic acid co-glycolide (70:30) were completely pulverized and then solvated in equal volumes of 50 mL of acetone and 50 mL of dichloromethane. The heparin and the polymers were then mixed and blended using intense high shear blending. After blending, 200 mL of deionized water was slowly added until opalescent microspheres formed. Blending was continued for an additional 10 to 15 minutes following the appearance of the microspheres, then the mixture was placed on a

pre-warmed hot-plate with a magnetic stirring rod. The temperature of the plate was 60° C for 12 hours.

When the granulate was completely dried, it was scraped from the filter material and passed through a 200 mesh sieve by gentle pressure until the dried granulate was homogeneous in size. The yield was 742 mg out of a theoretical yield of 1,400 mg. The resultant powder/granulate was weighed and titrated against protamine in normal saline to ensure that heparin was in fact entrapped in the spheres. To do this, the beads were suspended in normal saline and agitated by vortex. Protamine was then added to the solution and no precipitate was observed. The nanoparticles were assayed before degradation and found to have 0.75 U/mg of heparin.

Acetone and methylene chloride (2 mL) were added to disrupt the beads and, upon vortex, a heavy white paste precipitate was formed. The dry granulate thus produced was then evaluated and quantified and the product observed to contain 44.3 units of heparin per milligram of granulate. The powder was also examined for formation characteristics via a Phillips scanning electron microscope as shown in Figures 2A and 2B. Electron microscopy demonstrated an average diameter of 850 nm, with a range of 650 to 1250 nm.

To facilitate adsorption, the heparin-containing nanoparticles were coated with bioadhesive adjuvant. Nanoparticles were coated by dispersion in 20-mL of an aqueous adjuvant containing 0.5% Carbopol-934P and 0.5% hydroxymethylcellulose.

Example 2

Water Lipid Phase Sphere Formation of Vancomycin

Vancomycin hydrochloride (3,000 mg) was added to 300 mL of KH_2PO_4 at pH 6.8 and, using a high shear mixer, was blended with 3,000 mg of chitosan and 2,000 mg of pullulan and blended at high speed for 30 minutes. After a homogeneous opalescence appeared, 300 mg of zinc chloride was added to cross link the polymers. Then 30 mL of isopropyl alcohol was added to harden the spheres. Following this, 3,000 mg of liposome phospholin 90H, a prepared phospholipid from Natterman was added and homogenized for 15 minutes. The flask was then placed on a hot plate at the lowest temperature setting and magnetic stirring at 700 rpm's for 48 hours. After drying, the material was placed through a vacuum and 0.1 mm filter. When dry, the cake was removed and spread on a glass plate and allowed to dry at 60°C for 24 hours. The dried material was then screened through a 200 mesh screen. The theoretical yield was 12.0 grams while the actual weight after production 11.41 grams for 95.08% yield. The material was evaluated

via ultraviolet spectrophotometer and a standard curve developed. The dissolution showed the vancomycin content to be 0.64 mg/mL.

Example 3

Insulin and Cyclodextrin Nanoparticles

Ten thousand units of (100 mL) aqueous insulin was admixed with beta cyclodextrin (100 gm) and blended with a Silverson high-shear mixer until a clear solution developed, indicating entrapment of the insulin in the cyclodextrin. To this solution 500 mg of liposome phospholipon 90H was added and blended, then 1000 mg as a 30% W/V dispersion of methyl methacrylate was added. The material was spread on a special "petre" Pyrex plate and allowed to dry at 60°C for 4-6 hours until dry. It was then scrapped clean with a special razor knife and was so fine that screening was unnecessary. This material was placed in a test tube for later experimentation with diabetic rats.

Example 4

Water Procedure For Entrapment In Nanoparticles Insoluble In Organic Solvents

Where the biologically active molecule to be entrapped is insoluble, or may be destroyed by organic solvents, a water phase process may be used to incorporate the agent into spheres. In this example, a red lake dye, insoluble in organic solvents, was hydrated in an aqueous solution and precipitated with organic solvents. Red lake dye #40, 100 mg was suspended in 300 mL of ammoniated 0.05M KH_2PO_4 at pH 6.8 using a Silverson high shear mixer.

To this aqueous phase 3,000 mg of poly(3-hydroxybutyrate-covalerate 80:20) and poly DL-lactic acid was added and blended at high speed. Once the dye was suspended in the aqueous phase with the polymers, 1% polyvinyl alcohol W/W was added and blended with the materials as a stabilizing agent.

To this mixture was added an equal mixture of 30 mL of acetone and dichloromethane. Upon addition of the organic solvents an exothermic reaction occurred and there was the immediate formation of microparticles that were verified under light microscopy. The resultant solution was poured into 20 mL test tubes and centrifuged at 3000 g for 15 minutes. The supernatant was removed and the particulate material was repeatedly washed with deionized water until it was clear. The centrifuged material was then placed on a glass slide and allowed to air dry. After drying, there was a light pink powder that was fine and did not need screening.

The granulated material was placed into deionized water and vigorously vortexed for 15 minutes. No color was apparent. 5 mL of acetone and dichloromethane were placed with the beads and again vigorously vortexed. After this treatment, a bright red color appeared. This suggested that the method as explained entrapped the dye into the nanoparticles.

Example 5

Administration Of Oral Heparin In Nanoparticles

Experiment 1

Heparin nanoparticles, prepared according to Example 1, were orally administered to rabbits in an animal study. These preliminary results showed that heparin, a large molecular weight drug, can be formulated for oral absorption in a controlled, sustained-release fashion.

Fifteen adult rabbits were randomized as follows:

- 12 animals received oral heparin in nanoparticles at a dose of 2,400 U/kg of body weight;
- 1 animal received 11,000 units of plain liquid heparin;
- 1 animal received liquid 11,000 units of heparin and bioadhesive adjuvant alone; and
- 1 animal received only the bioadhesive adjuvant.

The rabbits were sedated with xyalazine for administration of the drugs and control. A soft #10 rubber French catheter was then placed into the animals' stomach and the material was delivered by pressure to simulate a controlled oral administration. The tube was rinsed with deionized water to make sure all the particles were expelled from the tube into the rabbits' stomach. In all, this process was repeated five times over a six month period with three different animals treated each time, for a total of 15 animals.

The animals were housed and fed rabbit chow and were given water *ad lib.* Blood samples were taken from the marginal ear vein at the following times, 6 hours post dose, 24, 48, 96, 120 and 144 hours after drug administration. The blood samples were placed in a citrate Eppendorf test tube immediately after blood was obtained and spun down using 3,000 Xg for 23 minutes. All samples were then assayed for antifactor Xa by Dr. Richard Malar at the University Of Colorado-Veterans Administration Hospital coagulation lab. The test was blinded as to the specimen, its timing and the specific animal. Following the centrifugation, the plasma was removed and frozen immediately at -20°C until assay.

The plasma was assayed for antifactor Xa using a standard, validated chromogenic method. The heparin-containing solution (50 µL) (at various dilutions and in duplicate) was added to a microtiter well in the presence of human plasma (50 µL) and factor Xa (50 µL). The reaction was incubated for 1 minute at room temperature and 50 µL of Chromozyme Xa substrate was then added. The mixture was incubated for 10 minutes at room temperature and the reaction stopped with 50 µL of 50% acetic acid. The assay well was then measured in an enzyme-linked immunoadsorbent assay reader at 405 nm. The amount of heparin was determined based on a standard curve; the duplicate values averaged and dilutions compared. The within-run variation was ±5% and the between-run variation was ±9%; the lower limit of detection was 0.01 U/mL. Values below <0.02 U/mL were considered as 0. The results are shown in Table 1 and Figure 3. The results of the three control animals show that there is no appreciable absorption of heparin under the test situations.

The results of this first experiment showed that heparin fashioned into nanoparticles produced sustained and viable plasma levels whereas regular heparin or the adjuvant given alone or together does not produce any significant levels of detectable heparin. Most importantly, this single dose produced effective doses for up to 144 hours after administration that was in stark contrast to injectable levels that seldom gave measurable levels of heparin 10-24 hours after subcutaneous injection of the drug.

Table 1 is a tabular display of animal studies with rabbits showing plasma heparin values (U/mL) after oral administration of heparin nanoparticles (2400 U/kg) (test animals); heparin only (11,000 U/kg); heparin (11,000 U/kg) plus adjuvant; or adjuvant alone (control) in 15 rabbits.

TABLE 1

Anti-Factor Xa Units of Heparin Following The Administration Of Heparin Nanospheres, Heparin, Heparin+Adjuvant, And Adjuvant

Units Of Heparin Per Milliliter U/mL

HOURS	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	MEAN	±SD	Heparin	H/ADJ *	ADJ*
0	0	0	0	0	0	0	0	0	0	0	0	0	0.00	0.26	0	0	0
6	0.9	1.38	1.1	0.7	0.7	0.8	0.9	0.7	0.31	0.7	0.5	0.8	0.79	0.67	0.01	0.02	0.08
24	0.12	3.04	0.8	1	1.3	0.7	1.1	0.9	0.6	1.3	0.8	1.2	1.07	0.35	0.02	0.02	0.01
48	0.25	0.1	0.9	1	1	0.8	0.9	1.1	0.35	1.2	1	1.1	0.81	0.56	0.07	0.01	0.02
72	0.9	0.29	1.1	0.8	0.8	2.3	1.3	1.6	0.16	0.8	1.3	0.6	1.00	0.23	0.09	0.03	0.01
96	0.8	0.36	1	1	0.8	1	0.7	0.8	0.3	0.6	0.5	0.9	0.73	0.45	0.08	0.08	0.04
120	0.6	0.37	2	0.9	0.9	0.8	1.2	0.5	0.17	0.6	0.7	0.7	0.79	0.21	0.12	0.01	0.01
144	0.7	0.31	0.8	0.9	0.7	0.7	0.6	0.4	0.3	0.5	0.2	0.5	0.55	0.21	0.09	0.02	0.03

* H/ADJ = Heparin + Adjuvant
* ADJ = Adjuvant Only

Experiment 2

Heparin nanoparticles, prepared as described in Example 1, were independently tested on an experimental mouse model that is sensitive to parenteral (injected) heparin. This experiment was performed in the research laboratories of Sanofi Pharma in France. Sanofi used a different animal species (mice) and different methods for evaluating heparin activity than that described in Experiment 1.

In this mouse model, a lethal dose of thromboplastin (100 mL/mouse), a blood clotting activating agent, was given intravenously 2 hours following various doses of drugs. The model then measures the level of protection given by the various drugs. The intravenous injection of thromboplastin with the dose of 100 µL/mice (0.2 ng) provokes a thromboembolism involving 80% mortality in 24 hours. The intravenous heparin standard protects 50% of animals treated when administered in the range of 0.09 mg/kg.

In this study, the heparin nanoparticles were given by oral route in suspension in a carboxymethyl cellulose solution (0.06%) in doses of 50, 150, and 300 mg/kg. In addition to the three dosage levels of the heparin nanoparticles tested, a blank and two heparin standards were run. The blank was the bioadhesive adjuvant, carboxymethyl cellulose, at 0.6%. The first heparin standard was intravenous heparin at 0.3 mg/kg. The second heparin standard was 85 mg/kg administered orally.

The results indicated in Table 2 show that the heparin nanoparticles protected 25% of mice treated with a dose of 150 mg/kg. The heparin standard had no effect in an oral dose of 85 mg/kg. The means of determining the effect of protection was done by subtracting the negative and positive controls from the test and determining the percentage of effectiveness.

It should be noted that the heparin nanoparticles have a total duration of measurable activity for 7 days and its peak effect is from 24 to 48 hours. Nonetheless, in this study, the activity was effectively measured only 2 hours after administration of the heparin by the injection of thromboplastin. Thus, the bias in the study is against the heparin nanoparticles. Protection was still found with heparin nanoparticles at 2 hours, which showed that the heparin nanoparticles produced an immediately active burst effect. In addition, the study showed that the heparin nanoparticles increased the intestinal adsorption of heparin.

Table 2

Treatment	Dose	Survival	% Protection
Vehicle	NG	2/10	20%
Heparin IV	0.3 mg/kg	8/10	80%
Heparin Nanoparticles	50 mg/kg	1/10	10%
	150 mg/kg	4/10	40%
	300 mg/kg	9/20	55%
Heparin liquid	85 mg/kg/po	2/10	20%

Example 6

Insulin Nanoparticles And Their Effect Upon Diabetic Rats

Since its discovery, insulin has been attempted to be given by means other than injection. The following shows the administration of insulin formulating according to this invention to animals in animal models.

Formulation

Insulin (10,000 units) as a concentrated liquid dosage was placed in a beaker and, using the Silverson mixer, blended at moderate speeds. Beta cyclodextrin was added at 1000 mg and blended with the insulin. Initially there was a milky white appearance that rather rapidly dissipated upon stirring. To this mixture was added a phospholipid at a strength of 500 mg. The agitation was continued until clearing occurred. At this point Eudragit® 30 RL at a 30% W/V solution was added (900 mg) and blending was continued once there was a homogeneous blend then 50 mL of ethanol was added to the mixture for the purpose of hardening the spheres. The fluid was then placed on a preheated stirring plate and at very low temperature <40°C and stirred 1700 Rpm's for 30 minutes. After stirring was complete, the fluid was placed into a modified Pyrex® dish and allowed to air dry for 24 hours. At the end of this time there was a very fine granulated powder that was removed by a sharp razor blade edge and weighed. The theoretical yield was 2.80 grams, while the actual yield was 2.75 grams.

The spheres were again vortexed in deionized water and protamine was added. There was no evidence of precipitation. However, when acetone and dichloromethane were added a heavy distinct precipitate occurred indicting that the insulin had been entrapped into the nanoparticles.

Animal Model

Three (3) test animals, rats made diabetic by streptozocin injection, were given varying amounts of the spheres with a bioadhesive. As this was a trial evaluation, only three animals were tested at this time. All animals had pretest blood sugars of +400 mg/dL. The results are demonstrated below. This experiment was performed by the veterinary unit at the Colorado State University School Of Veterinary Medicine.

Table 3

Blood Glucose

Rat#1	0 Hour	24 Hours	48 Hours	72 Hours	96 Hours
1092 units	414 ng/dL	212 mg/dL	44 mg/dL	125 mg/dL	386 mg/dL
Rat#2					
1092 units	444 mg/dL	420 mg/dL	126 mg/dL	157 mg./dL	328 mg/dL
Rat #3					
1092 units	427 mg/dL	312 mg/dL	110 mg/dL	134 mg/dL	470 mg/dL

Although the control and consistency were not as desirable as had been hoped for, the first trial was considered successful in that it was demonstrated yet again that a large molecule can be administered orally achieving physiologic activity over a prolonged period of time.

Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be obvious to one skilled in the art that certain changes and modifications may be practiced within the scope of the appended claims.

WHAT IS CLAIMED IS:

1. A controlled release pharmaceutical formulation comprising a biologically active molecule which is formed into nanoparticles with biodegradable hydrogel polymers and then coated with one or more bioadhesive adjuvants.

2. A controlled release pharmaceutical formulation comprising a biologically active molecule which is formed into nanoparticles with biodegradable hydrogel polymers, coated with liposomes, and then coated with one or more bioadhesive adjuvants.

3. A controlled release pharmaceutical formulation comprising a biologically active molecule encapsulated with a cyclodextrin, which is then formed into nanoparticles with biodegradable water-soluble hydrogel polymers, and then coated with one or more bioadhesive adjuvants.

4. A controlled release pharmaceutical formulation comprising a biologically active molecule which is encapsulated with a cyclodextrin, entrapped with liposomes and formed into nanoparticles then with water-soluble hydrogel polymers, and then coated with one or more bioadhesive adjuvants.

5. A controlled release pharmaceutical formulation comprising a biologically active molecule encapsulated with a cyclodextrin which is then formed into nanoparticles with biodegradable water-soluble hydrogel polymers, coated with liposomes, and then coated with one or more bioadhesive adjuvants.

6. The controlled release pharmaceutical formulation of claims 1-5, wherein said biodegradable hydrogel polymer is selected from the group consisting of poly (lactic acid), poly (glycolic acid), (poly (e-caprolactone), poly (p-hydrohybutyrate), poly (ß-hydroxyvalerate). poly (tartonic acid), polydixamone, poly (ethylene terephalate) poly (ortho esters), polyanhydrides, dextran, cross-linked polyvinyl alcohol, polyhydroxy methacrylate, polycyanoacrylates, poly (phosphoesters), polyphosphgenes, poly (hydroxy butyrate co-valerate), poly (2-hydroxyethyl glutamate), polyvinylpyrrolidone, poly (ethylene glycol), poly (propylene glycol), chitosan, pullulan, zein, alginic acid and alginate salts.

7. The controlled release pharmaceutical formulation of claims 1-5, wherein said biodegradable hydrogel polymer is selected from the group consisting of These include, but are not limited to acrylic acid, methacrylacetic cyanoethyl methocrylitic aminoakyl methacrylate copolymers, ethoxyethyl methacrylate copolymers, polymethacrylic acid, methacrylate acid alkylamide copolymer, polymethacrylic acid, polyacrylic acid, methacryic acid alkylamide copolymer, polymethacrylic acid (anhydride), and polymethyl methacrylate.

8. The controlled release pharmaceutical formulation of claims 3-5 wherein said cyclodextrin is selected from alpha, beta, and gamma cyclodextrins or a combination thereof.

9. The controlled release pharmaceutical formulation of claims 2, 4, or 5, wherein said liposomes are selected from the group consisting of cholesterol, lecithin, glycerol monostearate, phospholipon 90H (Natterman) maleated soybean oil, sphingomylein, phosphatidyl entanolamine, dipamitoyl phoisphophatidykaline, phophosinositol, phophatidic acid, triglycerides.

10. The controlled release pharmaceutical formulation of claims 1-5, wherein said bioadhesive adjuvant is a gum.

11. The controlled release pharmaceutical formulation of claim 10, wherein said gum is selected from the group consisting of pectins, guar gum, xantham gum, dextrin, gum acacia, gum dragon, and gum tragacanth.

12. The controlled release pharmaceutical formulation of claims 1-5, wherein said bioadhesive adjuvant is a cellulosic derivative.

13. The controlled release pharmaceutical formulation of claim 12, wherein said cellulosic derivatives is selected from the group consisting of hydroxypropyl cellulose, hydroxymethyl cellulose, hydroxypropyl methyl cellulose, hydroxypropyl alginate, and carboxymethyl cellulose.

14. The controlled release pharmaceutical formulation of claims 1-5, wherein said biologically active molecule is selected from the group consisting of a biological active protein, polypeptide, and/or polysaccharide molecule.

15. The controlled release pharmaceutical formulation of claim 14, wherein said formulation contains more than one biologically active molecule.

16. The controlled release pharmaceutical formulation of claim 14, wherein said biologically active molecule is heparin.

17. The controlled release pharmaceutical formulation of claim 14, wherein said biologically active molecule is insulin.

18. The controlled release pharmaceutical formulation of claims 1-5, wherein said formulation is administered by oral, parenteral or inhalation delivery.

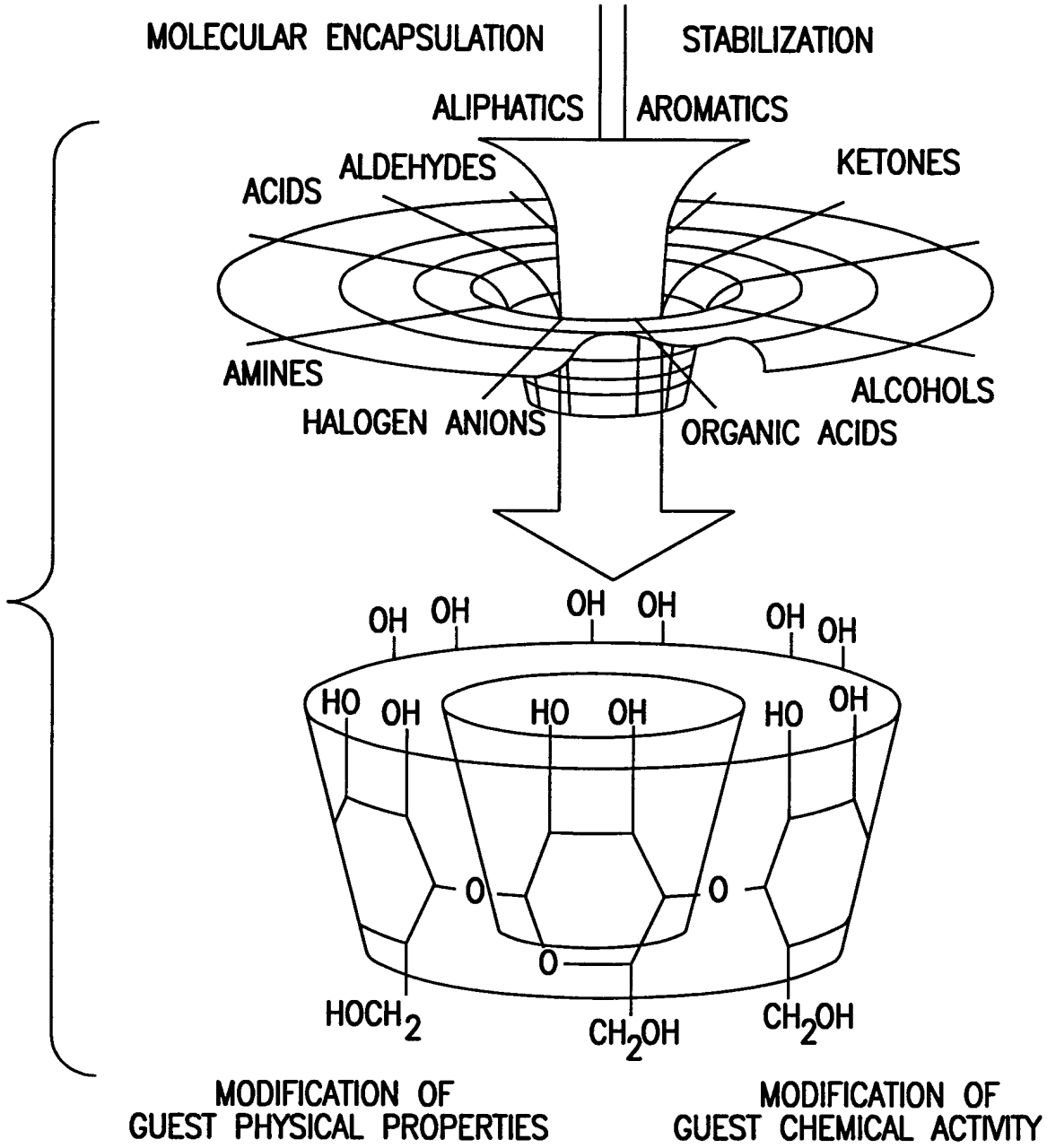
MOLECULAR ENCAPSULATION
STABILIZATION
ALIPHATICS
AROMATICS
ACIDS
ALDEHYDES
KETONES
AMINES
HALOGEN ANIONS
ORGANIC ACIDS
ALCOHOLS
OH
HO
O
HOCH2
CH2OH
MODIFICATION OF GUEST PHYSICAL PROPERTIES
MODIFICATION OF GUEST CHEMICAL ACTIVITY

FIG.1

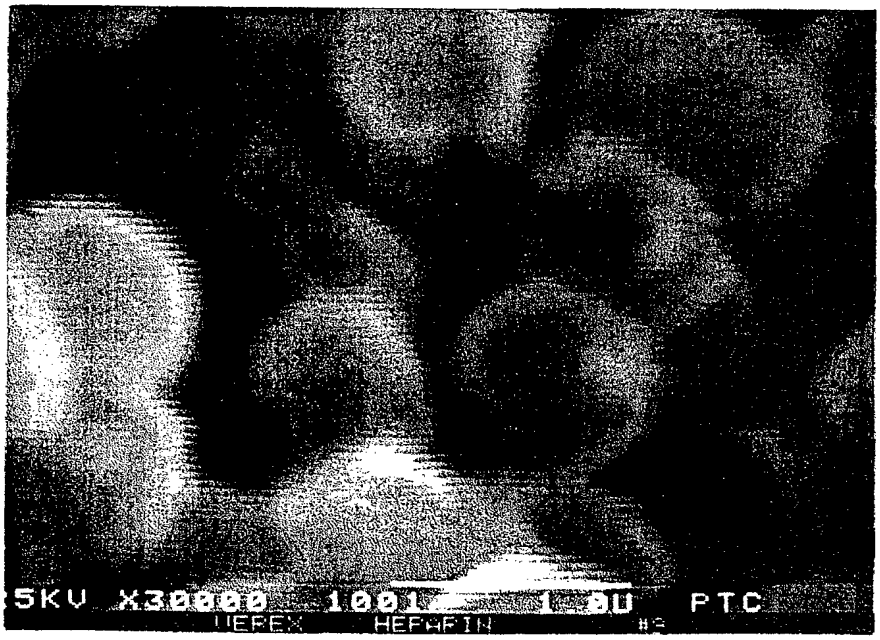
5KV X30000 1001 1.0U PTC
HEPARIN

FIG.2A

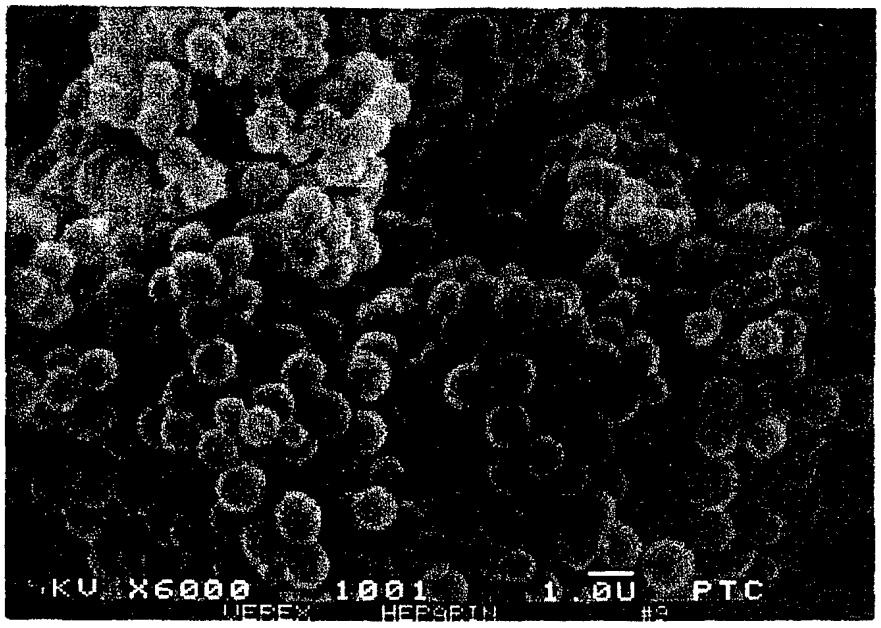
KV X6000 1001 1.0U PTC
HEPARIN

FIG.2B

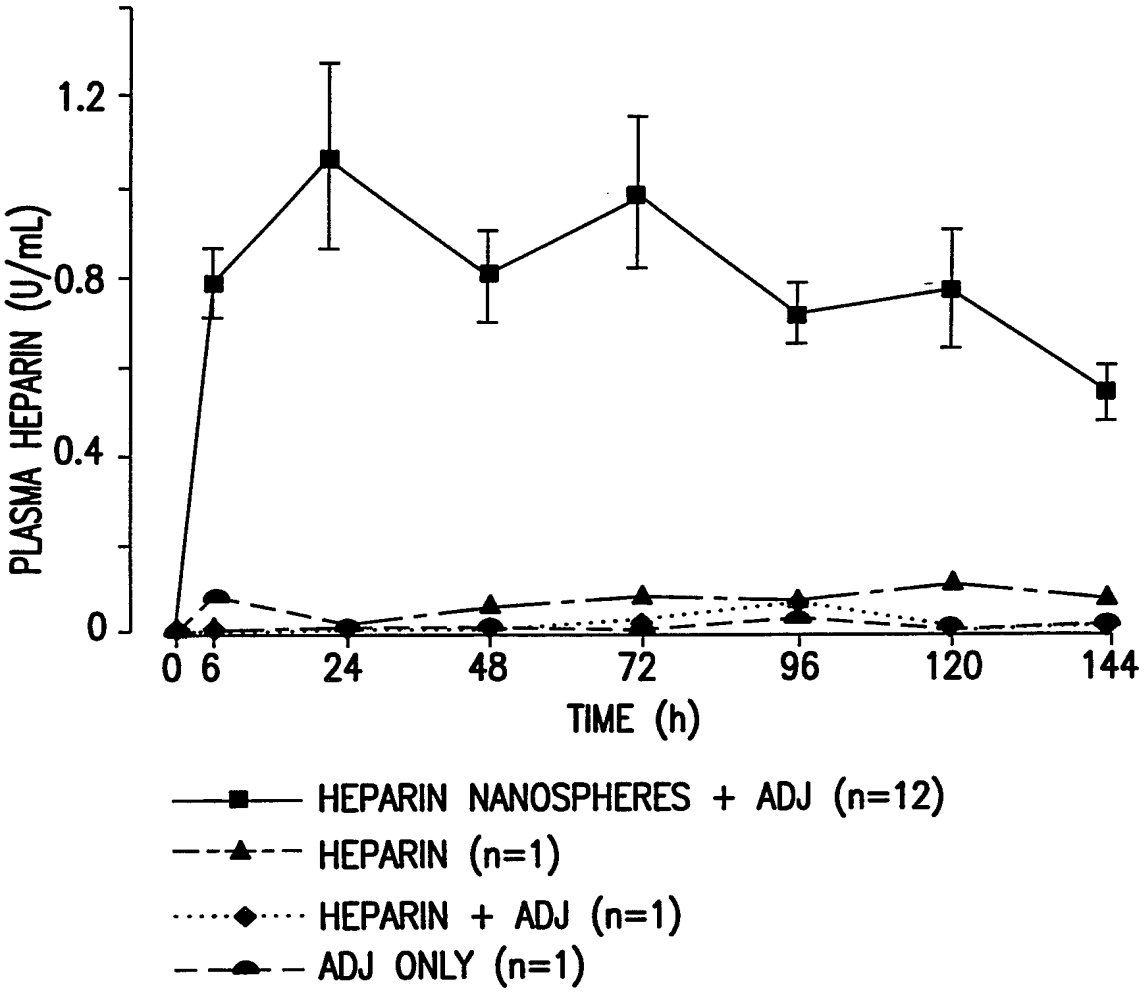
PLASMA HEPARIN (U/mL)
1.2
0.8
0.4
0
0 6 24 48 72 96 120 144
TIME (h)
HEPARIN NANOSPHERES + ADJ (n=12)
HEPARIN (n=1)
HEPARIN + ADJ (n=1)
ADJ ONLY (n=1)

FIG.3

INTERNATIONAL SEARCH REPORT

Int[illegible]ional application No.
PCT/US96/12203

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 9/127, 9/133, 9/16, 9/52, 9/58, 9/60
US CL :424/450, 489, 490, 491, 493, 494, 498, 499

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/450, 489, 490, 491, 493, 494, 498, 499

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

NONE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,417,982 A (MODI) 23 May 1995, abstract, columns 1-4, examples and claims.	1, 6, and 10-18
Y --- A	US 5,346,702 A (NA ET AL.) 13 September 1994, abstract, columns 1-2 and claims.	1, 6, and 10-18 --- 2-5 and 7-9
Y, P --- A, P	US 5,510,118 A (BOSCH ET AL.) 23 April 1996, abstract, columns 4-6 and claims.	1, 6, and 10-18 --- 2-5, and 7-9

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search	Date of mailing of the international search report
27 OCTOBER 1996	06 NOV 1996
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer G. S. KISHORE Telephone No. (703) 308-2351

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